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High CO_2 effects on postharvest biochemical and textural properties of white asparagus (*Asparagus officinalis* L.) spears

S. Huyskens-Keil[a,*], W.B. Herppich[b]

[a] Humboldt-Universität zu Berlin, Division Urban Plant Ecophysiology, Section Quality Dynamics/Postharvest Physiology, Lentzeallee 55/57, D-14195 Berlin, Germany
[b] Leibniz-Institute for Agricultural Engineering Potsdam-Bornim, Department of Horticultural Engineering, Max-Eyth-Allee 100, D-14469 Potsdam, Germany

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ABSTRACT

The effects of high CO_2 concentration (10% CO_2, 17% O_2) on the changes of functional cell wall components (pectic substances, hemicellulose, cellulose, lignin), mechanical properties, content of free soluble sugars (sucrose, glucose, fructose), and respiration activity were studied in harvested white asparagus spears stored at 10 and 20 °C, respectively, for up to 7 d. Spears stored at 2, 10 and 20 °C in air were studied as controls, where the 2 °C condition indicated the effects of cold storage. During storage, respiration activity declined only slightly, irrespective of the CO_2 and temperature regime. Spears stored at 20 °C under both CA and normal air became less stiff and more elastic, however, tissue toughness increased significantly. Changes in toughness were associated primarily with the dynamics of lignin and cellulose, revealing a strong correlation ($r^2 = 0.81$). High CO_2 concentration inhibited the synthesis of cellulose and, to some extent, lignin accumulation at 20 °C. Additionally, elevated CO_2 inhibited the degradation of soluble carbohydrates. In contrast, slightly lower temperatures of 10 °C in combination with high CO_2 did not have a pronounced effect on changes in structural carbohydrates (lignin, cellulose, hemicellulose and pectins). The effect low temperature (2 °C) under normal atmosphere conditions resulted in the inhibition of cell wall changes in asparagus spears.

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1. Introduction

White asparagus (*Asparagus officinalis* L.; Asparagaceae) is a highly nutritional (Maeda et al., 2005) and economically valuable vegetable. The spears of this monocotyledonous herbaceous perennial are succulent fleshy subterraneous shoots that grow from rhizomes. From the botanical view, they are "developmentally immature" (O'Donogue and Somerfield, 1998) and retain their high physiological activity (Heyes et al., 1998). Hence, spears show high rates of respiration (Papadopoulou et al., 2001), rapid degradation of soluble sugars (Lipton, 1990), proteins and ascorbic acid (Siomos et al., 2000), and pronounced water loss (Lipton, 1990).

Besides discoloration or microbiological decay, texture is the most crucial postharvest parameter that negatively affects quality and shelf-life of fresh and processed white asparagus spears. In a temperature-dependent manner (Rodríguez et al., 1999b; Herppich and Huyskens-Keil, 2008), spears become increasingly tough and fibrous (cf. Lipton, 1990; Siomos, 2003). These undesired changes result from further cell wall thickening (Chang, 1983; Zurera et al., 2000), increased lignification of cell walls of sclerenchyma sheath cells and of the vascular bundle elements (Billau, 1986; Waldron and Selvendran, 1990; Rodríguez et al., 1999c) or from a rapid increase in ferulic acid cross-linking of cell wall polymers (Rodríguez-Arcos et al., 2004; Jaramillo et al., 2007). All these reactions are assumed to be controlled by wounding-induced ethylene formation (Hsiao et al., 1981; Bhowmik and Matsui, 2004; Jaramillo et al., 2007). The textural changes may also simply reflect the unaltered continuation of shoot differentiation (O'Donogue and Somerfield, 1998; Herppich et al., 2005). It is also not clear whether secondary cell wall thickening is fed by a turnover of asparagus cell wall polysaccharides (Rodríguez et al., 1999c) or by a consumption of stored soluble sugars (Herppich and Huyskens-Keil, 2008).

Especially during storage or long distance transportation, low temperatures (0–2 °C) are generally recommended to retard produce quality losses and decay of asparagus (Lipton, 1990; Siomos, 2003). Low temperature diminishes water losses, reduces physiological activity and, concomitantly, respiration and consumption of soluble sugars (Chang, 1983; Lallu et al., 2000).

Because effective cool chain management is not always available during transportation and distribution (Lill and Corrigan, 1996; Renquist et al., 2005), controlled atmosphere (CA) or modified atmosphere packaging (MAP) may be successfully applied (Hurst et al., 1997; Kadau et al., 2003; Sothornvit and Kiatchanapaibul,

* Corresponding author. Tel.: +49 30 2093 46424; fax: +49 30 2093 46440.
E-mail address: susanne.huyskens@agrar.hu-berlin.de (S. Huyskens-Keil).

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2009). Comparable to low temperature, storage at high CO_2 and low O_2 is known to reduce physiological activity, thus diminishing respirational processes leading to an inhibition of energy supply for cell wall synthesis, spear toughening and changes in storage carbohydrate, protein depletion and asparagine accumulation (Everson et al., 1992; Siomos et al., 2000). However, investigations of combined CA/MAP and cold storage mostly report minor advantages over cold storage in air (Lipton, 1990; Siomos et al., 2000; Villanueva et al., 2005).

Postharvest variations in cell wall structure (Zurera et al., 2000) and composition (Redondo-Cuenca et al., 1997; O'Donogue and Somerfield, 1998; Rodríguez et al., 1999a,b,c; Kadau, 2005), and in soluble carbohydrates (Hsiao et al., 1981; Irving and Hurst, 1993; Huyskens-Keil et al., 2005; Kadau, 2005) of white asparagus spears have been investigated thoroughly. Moreover, the effects of cool and room temperature CA/MAP storage on cell wall polymers and textural properties of green asparagus have been also studied relatively extensively (e.g. Lipton, 1990; Waldron and Selvendran, 1990; Everson et al., 1992; Villanueva et al., 2005; Sothornvit and Kiatchanapaibul, 2009), but investigation on white asparagus are by far less frequent (Siomos et al., 2000; Jaramillo et al., 2007; Papoulias et al., 2009). Nevertheless, it has been shown that CA/MAP storage could largely inhibit spear toughening (Everson, 1992; Siomos et al., 2000; Villanueva et al., 2005). Variation of O_2 concentrations seems to be less effective on texture retention, but a CO_2 concentration of 15% seems to be optimal (Lougheed and Dewey, 1966). Everson et al. (1992) reported on a pronounced inhibiting effect of CA/MAP on lignin, which corresponds well with the finding that high CO_2 may probably inhibit phenylalanine ammonia-lyase (PAL, EC 4.3.1.1) (Holcroft and Kader, 1999), a key enzyme of lignin synthesis (Bhowmik and Matsui, 2004).

Despite all efforts, "the biochemistry and physiology underlying the beneficial effects of CA storage are not understood" (Hurst et al., 1997), especially concerning the effects of CA/MAP on asparagus spear toughening. It is still unclear whether CA inhibits singular physiological reactions or whether their decline is a response to a more general metabolic depression (Hurst et al., 1997). To better understand the underlying physiological processes, we studied the effects of high CO_2 concentration (10% CO_2, 17% O_2) during storage on potential respiration, content of free soluble sugars (sucrose, glucose, fructose) and the functional cell wall components (pectin, hemicellulose, cellulose, lignin) in spears stored at 10 °C (mimicking transport conditions) and 20 °C (simulating retail conditions), respectively, for up to 7 d. Asparagus stored at 2, 10 and 20 °C in air were also studied as controls (10 °C, 20 °C) and to indicate the effect of cold storage (2 °C) on biochemical and mechanical properties of white asparagus spears. This approach facilitates the comprehensive analysis of texture-related biomechanical and physiological targets in metabolic responses of this produce to high CO_2.

2. Materials and methods

2.1. Plant material and experimental design

Freshly harvested from a commercial field (Erzeugergruppierung Beelitz Spargel, Spargelgut Dietersdorf, Germany), white asparagus (*A. officinalis* L.) spears of the cultivar Gijnlim were transported to the laboratory, washed, sorted (according to EC quality standard class I), cut to a length of 22 cm (mean spear diameter: 1.8 ± 0.2 cm) and randomly separated into batches of approximately 500 g. Each batch of spears was placed loosely into a plastic container (30 cm × 40 cm × 5 cm) and fully covered with cloth, which was carefully soaked with demineralised water. In this water vapour saturated atmosphere, the spears were stored at 2 °C, 10 °C and 20 °C in air (0.03% CO_2 and 21% O_2), and at 10 °C and 20 °C in controlled atmosphere (10% CO_2, 17% O_2) for up to 7 d (3 repetitions, i.e. 3 batches each of approx. 500 g per day per treatment). For rapid and continuous CA adjustment, climate chambers (SB222, Weiss Umwelttechnik GmbH, Balingen, Germany) with a gas mixing unit and Ultramat CO_2/O_2 controller (Siemens AG, München, Germany) were used. Two independent experiments were performed.

2.2. Determination of respiration and mechanical properties

On the initial day of each experiment (day 0), 12 spears were used to evaluate the initial biological variability. On days 2, 4 and 7 of the experiment, six spears per treatment (two spears per batch) were randomly taken out of storage, equilibrated to room temperature (approximately 21.4 ± 0.9 °C) in water vapour saturated atmosphere for 1 h. Then, CO_2 release of all six asparagus shoots (on day 0 three mixed samples of four spears) was measured at 20 °C in closed Perspex cylinders with infra red sensors (FYA600CO2, Ahlborn Mess-und Regeltechnik, Germany). From the increase in CO_2 concentration over time and the spears' dry mass, respiration activity was calculated as mmol CO_2 g_{DM}^{-1} d^{-1}. Afterwards, fresh mass (*FM*, electronic balance BP 210 S, Sartorius AG, Göttingen, Germany), total length, and diameters at positions 2.5 cm, 7.5 cm, 12.5 cm and 18 cm from the base (electronic sliding calliper) were determined for each spear.

The acoustic impulse-response technique (Herppich et al., 2005) was then applied to determine the dynamic stiffness coefficient (*S*). Induced by slightly striking the spears with a little hammer in the middle section, the resulting sound signal was recorded with a microphone connected to the soundcard of a laptop (10 measurements per spear). From the first local maximum frequency (*f*) of the frequency spectrum, obtained after fast Fourier transformation of the raw sound signal, and the respective spear fresh mass, *S* was calculated as $S = f^2\ FM^{2/3}$.

Finally, on the positions 2.5, 7.5, 12.5 and 18 cm from base, the spears were sliced with a stainless steel microtome blade (S35, 0.26 mm total thickness, Feather Safety Razor Co. Ltd., Osaka, Japan) adapted to a Zwicki 1120 material testing machine (Zwick, Ulm, Germany; crosshead speed 600 mm min^{-1}) to obtain tissue toughness (Atkins and Vincent, 1984; Herppich et al., 2004), which is closely related to spear fibrousness (Vincent, 1990). Mean cutting force over the entire spear diameter (F_{cut}) and the actual cutting length (L_{cut}) were used to calculate the cutting energy ($E_{cut} = F_{cut}/(L_{cut} * \pi/4)$).

2.3. Analysis of carbohydrate content and chemical cell wall properties

On days 0, 2, 4 and 7, approximately 300 g of asparagus spears of each treatment were removed from the storage, freeze-dried, and thereafter subjected for further analysis of soluble mono- and disaccharides, and cell wall content of proteins, pectic substances, cellulose, hemi-cellulose and lignin.

After hot (70 °C) extraction of ground freeze-dried material (100 mg, three replicates) with ethanol (80%), the mono- and disaccharides (glucose, fructose and sucrose) were determined by High Performance Liquid Chromatography (HPLC Model 250, with RI-detector, 8110, Bischoff, Germany and Autosampler 708, Alcott, USA) over a water-spherisorb-amino column (250 mm × 3.0 mm, Bischoff, Leonberg, Germany) according to Ulrichs (1999). The mobile phase was acetonitrile and water (85:15) with a flow rate of 1 mL min^{-1}. Standard carbohydrate solutions (glucose 49140, Fluka, Neu-Ulm, Germany; fructose 5323, Merck KGaA, Darmstadt, Germany; sucrose 716260, Böhringer, Ingelheim, Germany) were prepared. The content of mono- and disaccharides was expressed as mmol g^{-1} dry mass.

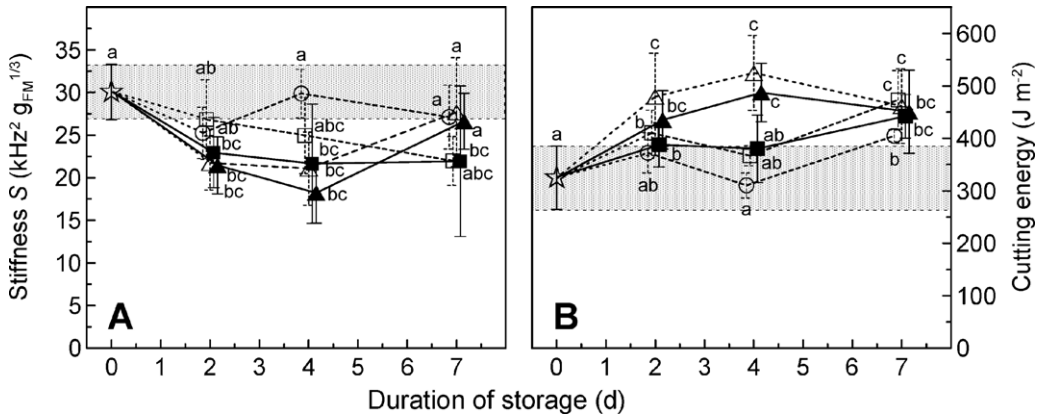

Fig. 1. Average shoot stiffness (A) and shoot toughness, indicated as the mean cutting energy (B) of white asparagus spears stored at 2 °C (○), and at 10 °C (□) or 20 °C in air (△), and in controlled atmosphere of 10% CO_2 and 17% O_2 (■, ▲) for up to seven days (means ± SD; $n = 6$). A star denotes the respective parameters of freshly harvested shoots (means ± SD; $n = 12$). Different letters indicate significant differences between means.

The alcohol insoluble fraction (AIF) was used to determine the cell wall protein content, modified according to the method described by Bradford (1976). Fifty mg of the dried, ground material was dispersed in 1 mL phosphate buffer with a vortex mixer (3 × 10 s), and afterwards centrifuged (11,400 rpm, 15 min; Multifuge X1, Heraeus, Hanau, Germany) at 4 °C. In a reaction tube, 100 μL of the supernatant were diluted with 900 μL phosphate buffer, 1 mL coomassie blue added and the carefully mixed solution measured photometrically (595 nm) after 20 min (PU 8730, Philips, Kassel, Germany). A calibration series (10–50 μg) was obtained from phosphate buffer and Bovine Serum Albumin.

Cell wall extraction for the determination of pectic substances (water soluble pectin, EDTA-soluble pectin and water insoluble pectin) was conducted according to Blumenkrantz and Asboe-Hansen (1973) modified by Huyskens (1991). The colourimetric determination of the pectin fractions was conducted using metahydroxybiphenyl (MHDP, Sigma H 6527, Sigma–Aldrich, München, Germany) as a colour reagent and following the method described by McComb and McCready (1952). In each fraction, the amount of galacturonic acid was measured photometrically (PU 8730, Philips, Kassel, Germany) at 520 nm. Analyses were performed with three replications for each treatment. The content of pectic substances was expressed as mg galacturonic acid g^{-1} dry mass.

Cellulose and lignin was analysed according to the methods of Van Soest and Goering (1963) and AOAC (1999). One gram freeze-dried sample was extracted with 100 mL Acid Detergent Fibre (ADF) reagent (N-Cetyl-N, N,N-trimethyl-ammoniumbromid dissolved with 96% H_2SO_4) using a Fibertec System (M 1020, Tecator, Sweden). Thereafter, the solution was vacuum filtered, washed with boiled, double distilled water until removal of the acidity and again washed with 90% acetone. The residue was dried at 105 °C for 24 h, weighed, ash-dried at 500 °C for 24 h and weighed again to calculate ADF. The dried ADF residue was used for Acid Detergent Lignin (ADL) determination. Cellulose content was calculated as the difference between ADF and ADL. The content of lignin and cellulose, respectively, were expressed as mg g^{-1} dry mass.

With the Neutral Detergent Fibre (NDL) approach (Van Soest and Goering, 1963) one gram of freeze-dried material was cooked in 100 mL of NDL mixture (Titriplex III, di-sodium borate, dodecylhydrogensulfate-Na, ethylene-glycol-monoethylester) to determine the hemicellulosic cell wall fraction. Afterwards, the solution was vacuum-filtered, washed with demineralised water and with 90% acetone. The insoluble residue was dried at 105 °C for 24 h, weighed, ash-dried at 500 °C for 24 h and weighed again to calculate NDF. The hemicellulose content was obtained by subtracting ADF from NDF and given as mg g^{-1} dry mass.

2.4. Data analysis and statistics

All data were statistically analysed (ANOVA) with WinSTAT (R. Fitch Software, Staufen, Germany). Treatments means were statistically compared using the Duncan's multiple range test ($P < 0.05$).

3. Results

3.1. Effects of storage conditions on mechanical properties and cell wall components

Irrespective of the storage conditions, spear stiffness declined during the initial storage period, i.e. until day 4 (Fig. 1A), although tissue water content (15.8 ± 1.3 g g_{DM}^{-1}) remained more or less constant (data not shown). Changes were smallest in spears stored at 2 °C and highest in those kept at 20 °C. High CO_2 concentrations seem to adversely affect stiffness both at 10 and 20 °C, though this effect was not significant. Although nearly all spears stored at 10 and 20 °C became less stiff during storage, their toughness increased significantly at 20 °C (Fig. 1B). At this temperature, storage at 10% CO_2 partially inhibited toughening. Reduction of temperature to 10 °C significantly reduced the increase in spear toughness, at least during the early storage. At 10 °C, CO_2 showed no effect. Spears stored at 2 °C in air, nearly retained their initial tenderness.

Mainly during the initial storage at 20 °C and, to a much smaller degree also at 10 °C, the spears' dry mass related contents of cellulose, lignin and hemicellulose significantly increased (Fig. 2A, C and E). At 20 °C, CA significantly limited the increase in the contents of these cell wall components; at 10 °C, this treatment was much less effective but still significant compared to storage in air in most cases. However, low temperature storage (0 °C) was much more effective and almost prevented the increase in cellulose, lignin and hemicellulose contents of spears.

Considering the relative contribution of the three major components to the overall cell wall material (Fig. 2B, D and F) may help to distinguish between accelerated deposition of a certain component and normal, coordinated cell wall growth. At 20 °C, high CO_2 storage clearly inhibited the excessive postharvest accumulation of cellulose and hemicellulose (Fig. 2B and F), restricting it to the same (Fig. 2F) or even below the fractions (Fig. 2B) observed at low temperature (2 °C). On the variation in the lignin fraction, however, the effects of CA treatments were only limited, both at 20 °C and at 10 °C (Fig. 2D). Furthermore, low temperature could nearly completely inhibit the relative accumulation of cellulose and hemicellulose but not that of lignin. Although the increase in the fraction

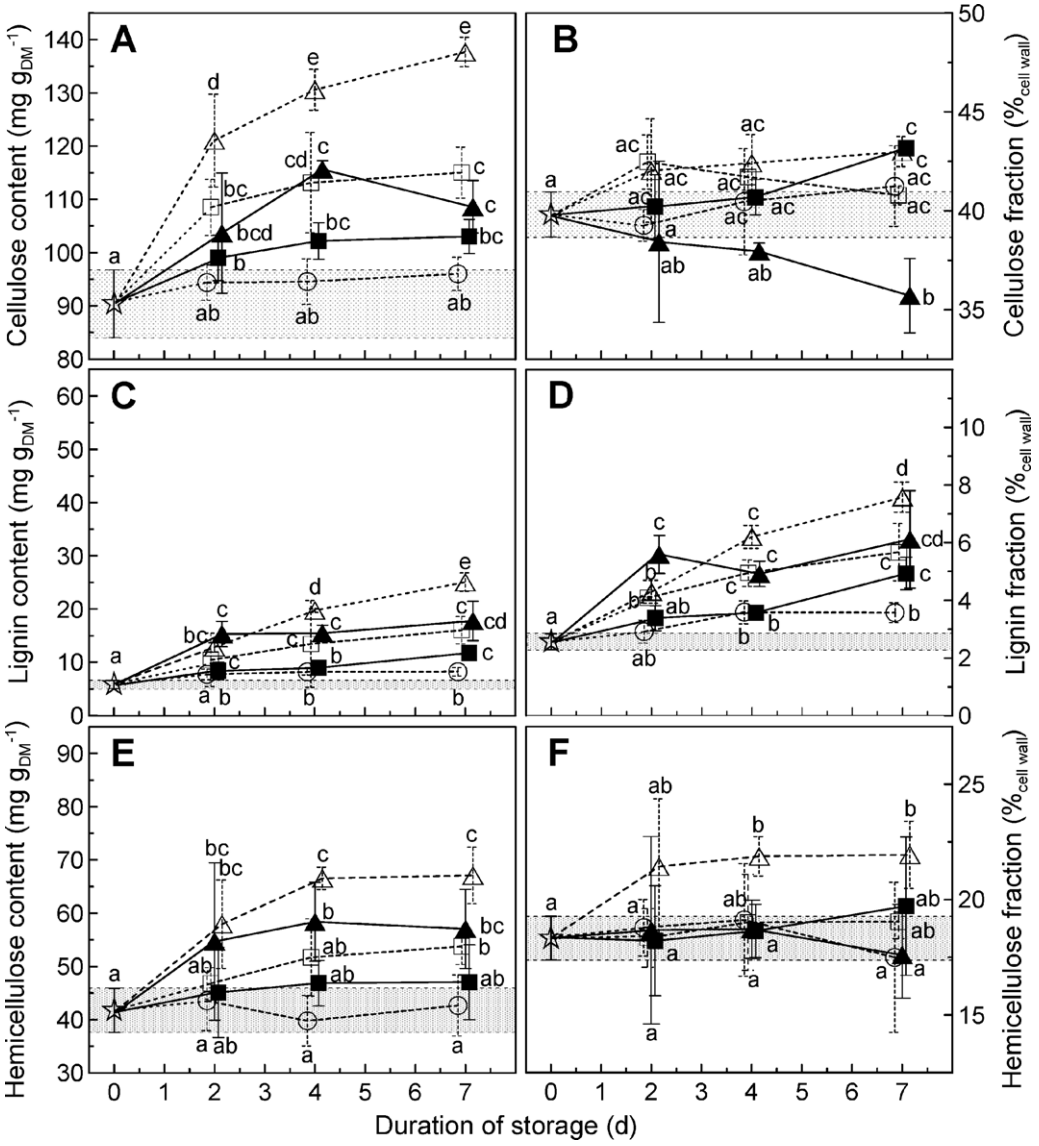

Fig. 2. Variation in the cellulose (A, B), lignin (C, D), and hemicellulose (E, F) content (A, C, E), and their relative contribution to total cell wall material (fraction; B, D, F) of cell walls isolated from white asparagus spears stored at 2 °C (○), and at 10 °C (□) or 20 °C in air (△), and in controlled atmosphere of 10% CO_2 and 17% O_2 (■, ▲) for up to seven days (means ± SD; $n = 6$). A star denotes the respective content of cell walls of freshly harvested shoots (means ± SD). Different letters indicate significant differences between means of two independent experiments.

of this cell wall component was small, the changes during storage were mostly significant. At moderately low temperature (10 °C), the combination with CA yielded nearly the same effect as storage at 2 °C.

A plot of the lignin content of all investigated samples against their cellulose content reveals the very close nonlinear (best fit obtained with a power function) relationship ($r^2 = 0.81$) between both cell wall components (Fig. 3). This may indicate that the dynamics of the changes of these cell wall components are equally affected by the different storage conditions. On the other hand, there is a tendency that CA treatment favours lignin accumulation compared to that of cellulose.

Pectic substances showed less pronounced changes (Fig. 4A and B) than the components involved in secondary cell wall thickening. Most changes of total and relative pectin content observed at 20 °C were due to an increase in water soluble (65%) and, to a smaller extent, of EDTA soluble pectins (26%) during late storage (data not shown). Only at 20 °C, could high CO_2 significantly reduce the accumulation of pectins. Moderately low temperatures (10 °C), however, already inhibited most accumulation of pectins, and storage at 2 °C prevented all changes.

The cell wall protein content of spears stored at 20 °C declined nearly linearly during the experiment (Fig. 4C). Cell wall protein degradation could be completely inhibited by high CO_2 concentrations and lower storage temperatures of 10 and 2 °C. However, the relative contribution of proteins to total cell wall declined with the duration of storage (Fig. 4D), except at a temperature of 2 °C. Nevertheless, CA could significantly reduce this effect although less pronounced at 10 °C compared to 20 °C.

3.2. Effects of storage conditions on soluble sugars and respiration

Irrespective of the treatments applied, both glucose and fructose contents of spears significantly declined during storage (Fig. 5A and B). The reduction of glucose was always double that of fructose. Cold storage (2 °C) and high CO_2 concentrations at 20 °C largely lowered sugar consumption (Fig. 5D). Sucrose content was much smaller than that of fructose or glucose (Fig. 5C). Except in spears stored

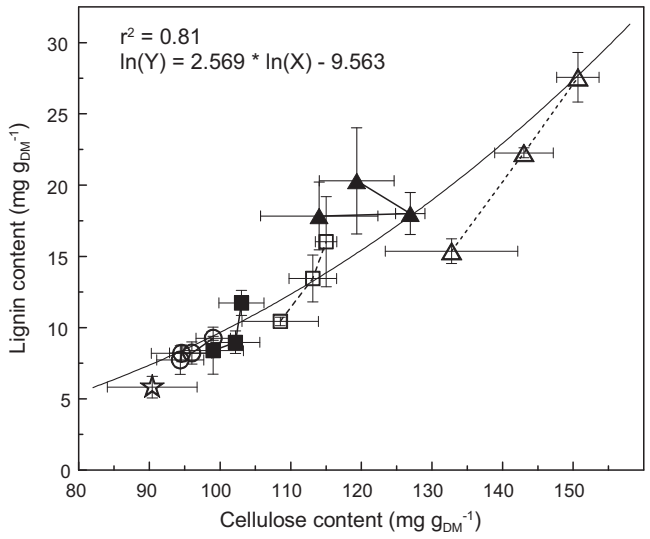

Fig. 3. Relationship between cellulose and lignin contents of cell walls isolated from white asparagus spears stored at 2 °C (○), and at 10 °C (□) or 20 °C in air (△) and in controlled atmosphere of 10% CO_2 and 17% O_2 (■, ▲) for up to seven days (means ± SD; $n = 6$). A star denotes the cellulose and lignin content, respectively, of cell walls of freshly harvested shoots (means ± SD).

in air at 20 °C, sucrose content increased during the experiments. This increase was higher the lower the storage temperature and was partially prevented by high CO_2. As a consequence of these changes, total contents of soluble sugars declined with storage duration under all conditions (Fig. 5D). The changes were less pronounced, the lower the storage temperature. Compared to free air storage, CA resulted in a significant reduction of sugar consumption only at 20 °C. Irrespective of the treatments, the variation of total contents of soluble sugars was highly correlated ($r^2 = 0.65$) with that of cell wall components (Fig. 6).

Respiration was 3.26 ± 0.36 mmol g_{DM1}^{-1} d^{-1} in freshly harvested spears, equivalent to a hexose consumption rate of 0.54 ± 0.06 $mmol_{glc}$ g_{DM}^{-1} d^{-1} (Fig. 7). Within two days of storage, respiration (measured at 20 °C in air) declined by 40%, 45% and 60% for spears stored at 2 °C, 10 °C and 20 °C, respectively, in air. High CO_2 storage affected respiration in spears stored at 20 °C (21% decline) but not when stored at 10 °C. During the rest of the experiments, respiration rates remained more or less constant under most conditions except for CA storage at 20 °C.

4. Discussion

4.1. Effects of high CO_2 storage on spear shelf-life

Elevated CO_2 concentrations in CA storage are known to reduce the metabolic activity of harvested products. In white asparagus spears, high CO_2 has been shown to suppress metabolic changes associated with the hydrolysis of sucrose and carbohydrate starvation, it also tendentiously reduced spear toughening (Everson et al., 1992; Hurst et al., 1997; Siomos et al., 2000). However, CO_2 concentrations above 15–20% might cause injuries in asparagus spears (Powrie and Skura, 1991; Sothornvit and Kiatchanapaibul, 2009). At higher than optimal temperature, theses harmful effects might even occur at CO_2 concentrations of 7% (Herner, 1987). Nevertheless, negative impacts of high CO_2 could not be approved for all quality attributes of white asparagus spears (Siomos et al., 2000). Therefore, it has been proposed as a suitable tool for maintain-

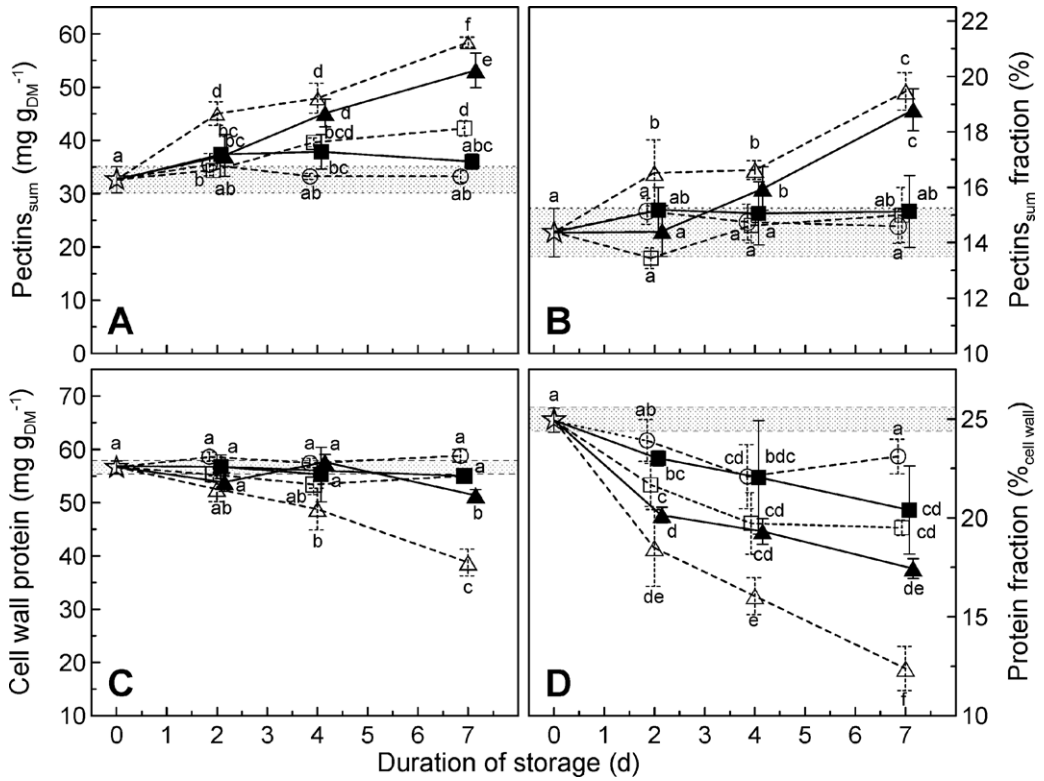

Fig. 4. Cell wall protein (A, B) and total pectin (C, D) content (A, C), and their relative contribution to total cell wall material (fractions B, D) of cell walls isolated from white asparagus spears stored at 2 °C (○), and at 10 °C (□) or 20 °C in air (△), and in controlled atmosphere of 10% CO_2 and 17% O_2 (■, ▲) for up to seven days (means ± SD; $n = 6$). A star denotes the respective content of cell walls of freshly harvested shoots (means ± SD). Different letters indicate significant differences between means of two independent experiments.

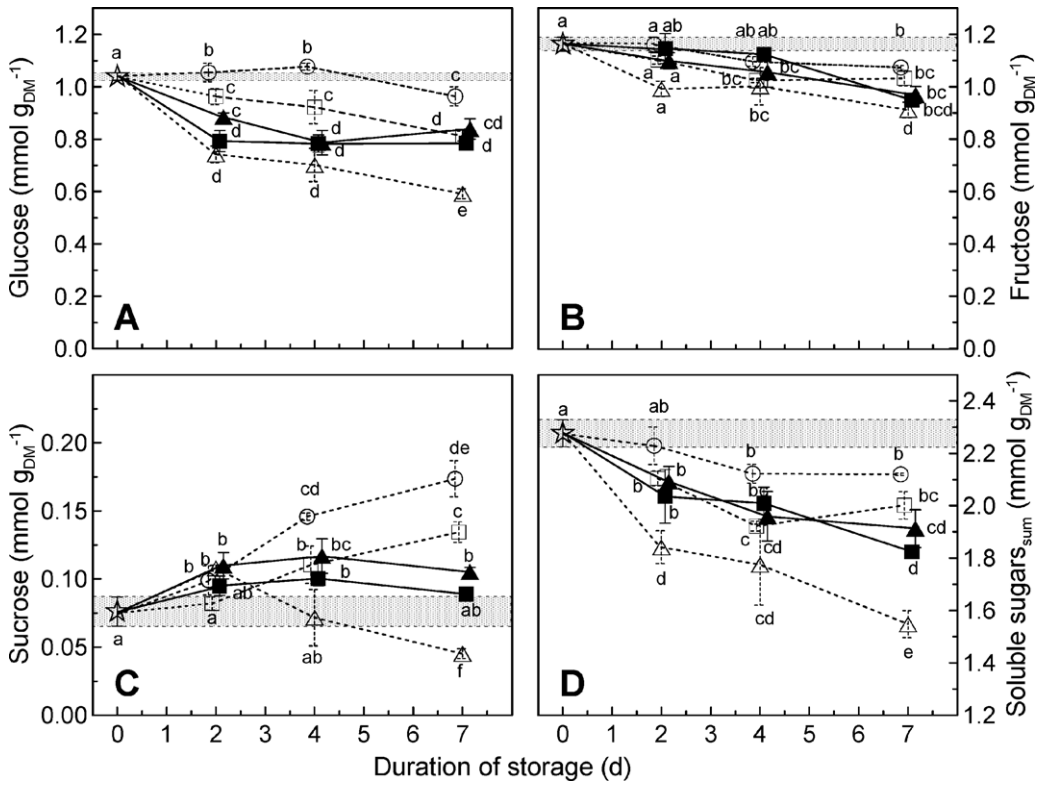

Fig. 5. Glucose (A), fructose (B) and sucrose content (C), and the sum of soluble sugars of white asparagus spears stored at 2 °C (○), and at 10 °C or 20 °C in air (□, △), and in controlled atmosphere of 10% CO_2 and 17% O_2 (■, ▲) for up to seven days (means ± SD; $n = 6$). A star denotes the respective content of cell walls of freshly harvested shoots (means ± SD). Different letters indicate significant differences between means of two independent experiments.

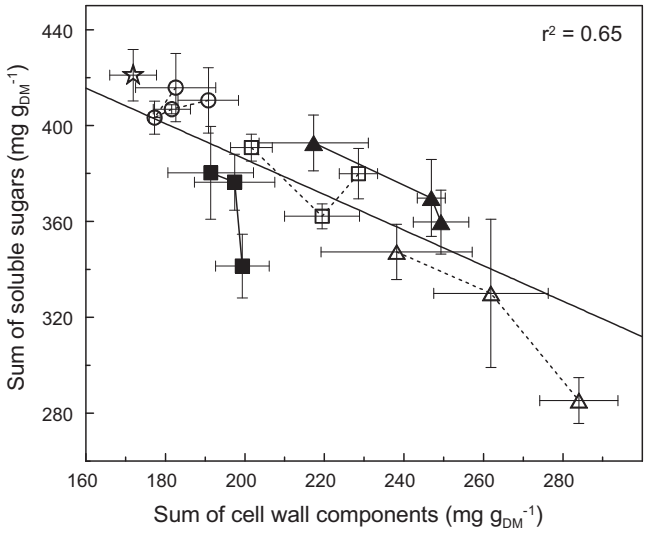

Fig. 6. Relationship between the total content of soluble sugars (glucose, fructose, sucrose) and the total content of structural components (cellulose, hemicellulose, pectins, lignin, protein) of cell walls of white asparagus spears stored at 2 °C (○), and at 10 °C (□) or 20 °C in air (△), and in controlled atmosphere of 10% CO_2 and 17% O_2 (■, ▲) (means ± SD; $n = 6$). A star denotes the respective values determined for freshly harvested shoots.

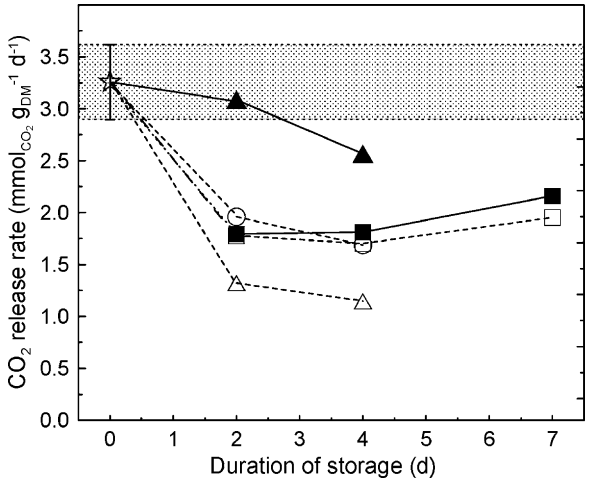

Fig. 7. CO_2 release rates of white asparagus spears stored at 2 °C (○), and at 10 °C (□) or 20 °C in air (△), and in controlled atmosphere of 10% CO_2 and 17% O_2 (■, ▲) for up to seven days. A star denotes the respective value determined for freshly harvested spears (means ± SD; $n = 3$), the other results were obtained on mixed samples of all six spears. All measurements were performed at 20 °C.

ing the textural properties of asparagus spears, and to markedly improve their shelf-life, at least under warm conditions (20 °C; Waldron and Selvendran, 1990; Lill and Corrigan, 1996; Renquist et al., 2005). However, the mechanisms behind these positive effects are still not fully elucidated.

4.2. *Effects of storage conditions on mechanical properties and cell wall components*

In general, texture is determined by both stiffness and toughness (Herppich et al., 2005). Stiffness provides the only means to non-destructively evaluate quality-related changes in texture properties. In contrast to toughness, the reduced stiffness, however, has only a negligible effect on sensory quality of asparagus spears, as also found in pervious experiments (Herppich et al.,

2005). Toughening of asparagus spears is generally related to the increase in both fibre and lignin content (Hsiao et al., 1981; Lipton, 1990; Siomos et al., 2000; Zurera et al., 2000). After harvest, cell growth and cell wall thickening, resulting from the highly active cell wall metabolism, lead to pronounced changes in cell wall mechanical properties (Waldron et al., 2003; Herppich et al., 2005). The development and thickening of secondary cell walls, mainly of sclerenchyma sheath cells and of the vascular bundle elements, is closely reflected by the increase in fibre content (Waldron and Selvendran, 1990). On the other hand, secondary thickening is associated with the augmentation of cell wall lignification (Mellerowicz et al., 2001; Anderson-Gunneras et al., 2005). Enhanced incorporation of lignin is important for the structural integrity of cells, and the rigidity and the strength (toughness) of stems (Boerjan et al., 2003). Hence, increased lignification of pericyclic fibres has been assumed to be the major cause of toughening (Siomos et al., 2000; Bhowmik et al., 2003).

However, as indicated by the present results, changes in toughness in asparagus spears were closely associated with the dynamics of both major secondary cell wall components, i.e. lignin and cellulose (Albersheim et al., 2011), and their contribution to total cell wall material. In contrast, pectic substances and proteins (Lamport et al., 2010), which are major components of primary cell walls, contributed only to a low extent to the toughening processes. Changes in pectic substances were also found to be predominantly temperature mediated and less influenced by elevated CO_2 concentrations. Furthermore, the relative contribution of proteins to the entire cell wall material even declined with storage duration (Herppich and Huyskens-Keil, 2008). This may be interpreted as a kind of overall "dilution" of this component by secondary cell wall material. Some structural proteins have been identified to strengthen the cell wall, among others, in response to osmotic (Marshall et al., 1999) or cold (Gómez Galindo et al., 2004) stress. The reduction in the protein fraction, which was enhanced by temperature and reduced by the elevated CO_2 concentration, may indicate that these structural elements do not significantly contribute to shoot strength or toughness.

Thus, in asparagus spears, toughening related modifications occur in the secondary cell walls, which further points out the major role of cellulose and lignin in this context (Saltveit, 1988; Everson et al., 1992; Bhowmik and Matsui, 2004; Villanueva et al., 2005). From the presented results, it is obvious that the increase in the contents of these cell wall components is low at low storage temperatures. This directly indicates a partial or full inhibition of thickening and toughening of cell walls by spear cooling, as has also been reported by others (Rodríguez et al., 1999b; Zurera et al., 2000). Furthermore, temperature dependence of the variation in cellulose and lignin content were nearly identical (Herppich and Huyskens-Keil, 2008), despite the large difference in their overall contents.

Although low temperature more pronouncedly affected spear toughening than did high CO_2, the presented results verified earlier reports that CA or MAP may partially inhibit this effect at room temperatures but much less effective during cold storage (Everson et al., 1992; Villanueva et al., 2005; Chen et al., 2009; Tu et al., 2009). At 20 °C, high CO_2 is assumed to generally suppress metabolic processes (Lill and Corrigan, 1996; Hurst et al., 1997; Renquist et al., 2005), which also includes cell wall development and growth. However, it is not clear whether there is a direct effect of high CO_2 on lignification.

In the present study, high CO_2 partially inhibited postharvest synthesis of all cell wall components to more or less the same extent. This may indicate that both the rapid polymerisation and epimerisation of cell wall microfibrils, leading to the thickening of the cell walls, and lignification was retarded by elevated CO_2 to nearly the same extent both at 10 °C and 20 °C.

In a very species-specific manner, elevated CO_2 may have inhibitory, no or even stimulations effect (Davey et al., 2004; Luo and Polle, 2009). Nevertheless, a reduction in lignin synthesis in response to high CO_2 in asparagus spears is an attractive assumption. It corresponds well with the finding that elevated CO_2 partially inhibits PAL activity (Holcroft and Kader, 1999; An et al., 2007; Chen et al., 2009).

It is assumed that lignification, and also thickening, is controlled by ethylene, directly influencing PAL activity (Mathooko et al., 1995). Wound stress-induced by cutting at harvest (Papadopoulou et al., 2001; Bhowmik and Matsui, 2004; Jaramillo et al., 2007), ethylene is a general response of plant tissues to injuries through an activation of ACC synthase and ACC oxidase (Kato et al., 2000). These processes might be prevented by elevated CO_2 concentrations (Siomos et al., 2010). Thus, stress-mediated enhancement of ethylene production, which, in turn, induces lignin formation via PAL, is known to have a detrimental effect on toughening in harvested asparagus spears (Bhowmik and Matsui, 2004; Liu and Jiang, 2005). However, there are also experimental findings that wounding does not accelerate ethylene production of asparagus (Hennion and Hartmann, 1990).

Moreover, it was shown that CO_2 can promote (Siripanich and Kader, 1985), inhibit (Chen et al., 2009; Siomos et al., 2010) or has no effect (e.g. Assis et al., 2001) on ethylene action and PAL activity. These contrasting findings stress that CO_2-mediated effects are highly dependent on species, physiological age at the time of exposure, CO_2 concentration and duration of exposure. Furthermore, the fact that only lignin continued to increase during storage, irrespective of the treatment, may rule out any direct or specific effect of elevated carbon dioxide on this cell wall component. In this context, Tsoumaki et al. (2009) showed that MAP reduced toughening but not lignin synthesis in cold stored white asparagus.

4.3. Effects of storage conditions on respiration, soluble sugars and their interactions with cell walls

Among fruit and vegetables, asparagus spears have the highest initial rate of respiration (Brash et al., 1995; Siomos, 2003). High CO_2 concentrations are, in general, known to reduce respiration rates in many horticultural products (Kays and Paull, 2004). During the entire experimental storage period, respiration declined more or less irrespective of CO_2 regime and temperature. This might be due to the fact that no stress-(wound-) induced, ethylene-mediated increase in respiration occurs during shelf-life of asparagus spears (e.g. Hennion and Hartmann, 1990; Papadopoulou et al., 2001). Respiration rates of asparagus vary with temperature and time elapsed from harvest, being highest immediately after harvest and dropping to a constant rate during postharvest (Lipton, 1990).

In the present study, high CO_2 concentrations only at ambient temperature and not at low temperature (10 °C) alleviate sugar consumption. Thus, at high temperature, CA conditions suppressed respiration leading to a partial inhibition of energy supply for spear toughening, cell wall synthesis, and changes in storage carbohydrates to a certain extent (Renquist et al., 2005). The minor increase in sucrose content and the reduced consumption of reducing sugars, observed at cool (10 °C) and cold (2 °C) storage temperatures, might indicate their decreased need as a carbon source for structural carbohydrates. Postharvest fibre accumulation is mainly attributed to the consumption of stored soluble sugars (Herppich and Huyskens-Keil, 2008) or to a turnover of primary cell wall polysaccharides (Rodríguez et al., 1999c).

Furthermore, a minimum hexose consumption rate of 0.19 $mmol_{glc}\ g_{DM}^{-1}\ d^{-1}$, observed in spears stored at 20 °C, should yield a total of 1.34 $mmol_{glc}\ g_{DM}^{-1}$ within the duration of experiment, while total change in soluble sugar was only 0.73 $mmol\ g_{DM}^{-1}$. Hence, normal respiration measurements

tended to overestimate respirational sugar consumption. On the other hand, a comparison of the variation of sugar content and cell wall components revealed a close correlation between each other. This may indicate that the synthesis of the cell wall components is the main sink for free soluble carbohydrates and not respiration.

Reports have indicated that the rapid loss of sugars (in the present study glucose and fructose) is an early and significant change in asparagus spears (Irving and Hurst, 1993). Low sugar concentrations may be involved in signalling for senescence gene induction (Coupe et al., 2002). There are various reports on changes in the carbohydrate pattern of green asparagus during cold storage indicating an increase in glucose and xylose and decline in arabinose and galactose contents (e.g. Waldron and Selvendran, 1990; Rodríguez et al., 1999a; Villanueva-Suarez et al., 1999). All these findings may be attributed to the CO_2- and temperature-mediated inhibition/acceleration of the enzyme activities in asparagus being linked to sucrose degradation but also act in the direction of sucrose synthesis (McKenzie et al., 2004).

5. Conclusion

The interactive effects of high CO_2 (10% CO_2) and temperature on the dynamics of toughness, functional cell wall components, respiration and soluble carbohydrate of white asparagus spears during storage (up to 7 d) was investigated to evaluate the respective potential control of high CO_2 and temperature on the underlying physiological processes.

Elevated CO_2 storage tended to reduce toughening only at ambient temperature (20 °C). At 20 °C and, to lower extent, at 10 °C, high CO_2 inhibited the synthesis of secondary cell wall components (hemicellulose, cellulose and lignin). Primary cell walls elements were less affected (pectins) or their content even reduced (cell wall proteins) during storage. Compared to structural carbohydrates, inhibitory high CO_2 effects on lignin formation were less pronounced. Hence, a prominent role of lignin in spear toughening seems to be disputable.

Except for pectins, the inhibitory effects of elevated CO_2 on cell wall synthesis were similar to those obtained under lower temperature (i.e. effects of high CO_2 storage at 20 °C ≈ reduction of temperature to 10 °C; effects of high CO_2 storage at 10 °C ≈ reduction of temperature to 2 °C). Only at room temperature (20 °C), high CO_2 directly affects respiration capacity. As a consequence, consumption of soluble sugars is significantly reduced by CA conditions only at this temperature.

Thus, high CO_2 seems not to specifically target single physiological reactions; high CO_2 effects on toughening, however, result from a more general metabolic depression, i.e. the reduction of various anabolic and catabolic processes, and their interactions.

The results verify that CA or MAP is a valuable tool for quality maintenance in white asparagus if no continuous refrigerated storage is possible. Nevertheless, low temperatures (e.g. 2 °C) are more effective.

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